THE AMMONIA TOLERANCE TEST IN HORSES

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ABSTRACT

Clinically normal horses (n=8) with ages ranging from 5 to 8 years, were starved for 12 h and their plasma ammonia concentrations were measured. The mean fasting plasma ammonia concentration was 17,8 ± 3,8 μmol ℓ^{-1}. After dosing ammonium chloride at a dose rate of 0,02 g kg^{-1}, there was a significant increase in plasma ammonia concentration, with a maximum rise after 20 min ($P < 0,05$). To investigate the influence of temperature on plasma ammonia concentrations of stored samples, 8 plasma samples were stored at -20°C and 4°C respectively. The plasma ammonia concentrations were measured after 6, 12 and 24 h in each of the stored samples. Plasma ammonia concentrations increased significantly after 12 and 24 h when stored at 4°C ($P < 0,05$). When plasma was stored at -20°C there was no significant increase from baseline concentrations during 24h ($P > 0,05$).

Key words: Plasma ammonia, ammonia tolerance test, horses

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INTRODUCTION

Detection of chronic liver damage in the horse has its limitations due to the fact that the clinical pathologist is limited to the sulfobromophthalein (BSP) clearance test, clotting factors, plasma protein, blood ammonia and bile acid concentrations[5]. However, as pharmacological grade BSP dye is no longer available, other methods of assessing liver function should be investigated.

Plasma ammonia is produced by microbial deamination of urea and exogenous dietary amines in the intestinal tract[13]. Ammonia is absorbed from the intestine and carried via the portal venous blood to the liver where it is converted to urea[13]. When hepatic function is severely impaired, or when collateral communication between portal and systemic veins develops, ammonia concentrations may increase in systemic blood[4].

Plasma ammonia concentrations in normal horses and in horses with chronic liver disease, are well-documented in the literature and summarised in Table 1.

Table 1: **Reference values for plasma ammonia concentrations in equines**

Reference	Normal (μmol ℓ^{-1})	Chronic liver disease (μmol ℓ^{-1})
1	18,3-22,4	57,1-374,0
3	7,7-63,7	64,3-454,3
8	68,7	309,0
11	68,7	76,7-164,0
12	5,2-7,3	11,2
16	15,6	--
17	5,0-9,7	227,4-308,6
18	83	431,0

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of blood around the liver[4]. With 60% or more loss of hepatic mass in the dog, the plasma ammonia concentration may remain within normal limits. However the post-dosing plasma ammonia concentrations of these dogs will be about 5 times the baseline concentration[4].

The toxic effects of ammonia in the horse are well-documented[9] and hepatic encephalopathy is a well-recognised disease entity in equines[17 18]. By dosing urea at 450 g per pony, 7 out of 8 ponies died within 12 h[9]. The lethal dose of ammonia for farm animals is 0,5-1,5 g kg^{-1} and clinical signs of toxicity can be seen with a minimum dose of 0,3 to 0,5 g kg^{-1} in the horse[13]. Care should therefore be taken in dosing ammonia to horses with high basal concentrations of ammonia.

The collection, handling and storage of samples for ammonia determination have received considerable attention[10 15 16]. Blood samples should be collected in ammonia-free heparin and the plasma separated from the blood cells within 30 min[16]. The plasma can then be stored at 4°C for a maximum period of 2 h before the ammonia concentration is determined. Anticoagulants such as sodium citrate, potassium oxalate and sodium fluoride will give erroneously high results[16], but no studies have been done on EDTA plasma for ammonia determination in the horse. EDTA was the anticoagulant recommended by the company marketing the enzymatic UV test reagents for ammonia determination, used in this trial.

Meyer et al.[15] showed that by performing a standardised ammonia tolerance test, normal dogs could be clearly separated from dogs with portosystemic shunts. The ammonia tolerance test in dogs is performed by fasting the animals for 12 h[15]. A baseline plasma sample is collected before dosing with 20% ammonium chloride solution at a rate of 0,1 g kg^{-1} per os[4 15]. A 30 min post-dosing plasma sample is obtained for ammonia assay[4 15]. In healthy dogs, the post-dosing plasma ammonia concentration is about 2-2,5 of the baseline concentration[4]. Higher values than this, indicates reduced functional hepatic mass secondary to shunting

The objectives of this trial were to establish baseline values for the ammonia tolerance test in clinically normal horses and to investigate the influence of temperature on the plasma ammonia concentration in stored samples.

MATERIALS AND METHODS

Apparently clinically normal, Thoroughbred geldings (n=8) were used in this trial. Their ages ranged from 5 to 8 years. All horses were housed individually and fed a mixture of lucerne and teff unless

otherwise stated. To ensure that the liver function in each of the horses was normal, total serum protein, albumin and globulin concentrations were measured in each horse. Only horses with albumin concentrations exceeding 30 g ℓ^{-1} were included in the trial. One gram of sulfobromophthalein (BSP) was injected intravenously and blood samples were collected in heparin from the opposite jugular vein after 4 and 9 min. The BSP concentration was measured and plotted against a standard curve on semilog paper and the T 1/2 calculated. Only horses with a T 1/2 for BSP of less than 3,8 min were included in the trial. The normal T 1/2 for BSP excresion in horses is 3,8 min[2 3 14].

Each horse was starved for 12 h and then weighed. Venous blood was collected in EDTA and centrifuged at 4 000 r.p.m. (Roto - uni II, Optolabor) for 5 to 10 min as soon as possible after collection. The plasma was separated and analysed for ammonia within 2 h. The plasma ammonia concentration was determined, using an enzymatic UV method with Boehringer Mannheim GmbH reagents (Boehringer, Mannheim, West Germany) and an LP6 spectrophotometer (Dr Lange). Each sample was analysed in triplicate.

The remainder of each plasma sample was split into 2 groups of 3 × 1 ml aliquots and stored in capped plastic tubes at 4°C and -20°C respectively. A sample from each group was analysed in triplicate for ammonia concentrations after 6, 12 and 24 hours to determine the effect of storage on ammonia concentrations.

If the basal ammonia concentrations were within acceptable limits (below 80 μmol ℓ^{-1}) 0,02 g kg^{-1} ammonium chloride in a 20% solution was dosed via stomach tube to all the starved horses. The horses were allowed free access to feed after dosing. Post-dosing blood samples were obtained every 10 min for a period of one hour and processed as described above.

Analysis of variance was used to determine if there was a significant change in the measured ammonia concentrations after dosing and after storage. A 95% confidence interval was regarded as significant. Student's t test with a 95% confidence interval was used for testing significance of the baseline ammonia concentration and the increase after 20 min. Results were reported as mean ± standard deviation.

RESULTS

The mean fasting plasma ammonia concentration in the horses (n = 8) was 17,8 ± 3,8 μmol ℓ^{-1}. There was a significant increase in plasma ammonia concentration after dosing ammonium chloride with the maximum increase after 20 min ($P < 0,05$). Post-dosing plasma ammonia concentration are summarised in Table 2. Plasma ammonia concentrations increased significantly from baseline levels after 12 and 24 h when stored at 4°C ($P < 0,05$). When plasma was stored at -20°C there was no significant increase from baseline concentrations after 24 h ($P > 0,05$). The changes in plasma ammonia concentrations after 6, 12 and 24 h are summarised in Table 3.

Table 2: **Plasma ammonia concentrations in 8 horses after ammonium chloride administration**

Time (min)	Ammonia concentration (μmol ℓ^{-1})	Range (μmol ℓ^{-1})
0	17,8 ± 3,8	10,1 - 23,9
10	43,7 ± 43,3	13,8 - 141,8
20	92,7 ± 72,9	18,1 - 230,2
30	58,6 ± 46,0	15,8 - 160,0
40	28,7 ± 13,5	17,0 - 57,3
50	25,2 ± 6,8	17,0 - 38,6
60	24,3 ± 5,4	18,1 - 32,0

DISCUSSION

The fasting plasma ammonia concentrations measured in this trial are considerably lower than the concentrations reported by others[8 12 18]. Since diet can influence the concentration of ammonia production in the intestinal tract and subsequent absorption and transport to the liver, it is therefore important to compare fasting plasma ammonia concentrations with reference values[4].

Plasma ammonia concentrations increased significantly after dosing ammonium chloride at a dose rate of 0,02 g kg^{-1} orally to starved horses. This dose rate is considerably less than the one used by Evans et al.[6] where ammonium chloride was used to acidify urine in horses. If an ammonia tolerance test is done when the baseline plasma ammonia concentration is high, consideration should be given to the possible pathophysiological consequences of hyperammonemia[4].

The post-dosing plasma ammonia concentration of 92,7 ± 72,9 μmol ℓ^{-1} (at 20 min) in this trial is higher than the post-dosing increase reported in dogs[4 15]. This may be due to the post-dosing sample in dogs only being collected after 30 min[15]. The ammonia tolerance test is used to diagnose congenital portocaval shunts and acquired shunts due to chronic fibrosing liver disease in the dog[4]. According to the opinion of Engelking et al.[5] based on unpublished data, the ammonia tolerance test in horses showed promise as a diagnostic aid for detecting hepatic failure[5].

Plasma ammonia concentrations may decrease during storage, due to vaporous loss as equilibrium is established between aqueous and gaseous phases[10]. Hemolysed blood samples should be rejected because erythrocytes have ammonia concentrations 2,8 times higher than that of plasma[7]. Animals should be fasted at least 6 h before sample collection, because the ingestion of protein has been shown to increase plasma ammonia concentrations[16].

Results of this study showed that ammonia concentrations in plasma will increase significantly when stored at 4°C, which is in support of the results reported by Ogilvie et al.[16]. This may occur due to

Table 3: **Changes in plasma ammonia concentrations (mean ± SD) after storage for 6, 12 and 24 h**

		Plasma ammonia concentration* after: (μmol ℓ^{-1})		
Temperature	**n**	**6 h**	**12 h**	**24 h**
4°C	8	+ 4,6 ± 3,5	+ 8,9 ± 4,7	+ 11,4 ± 3,3
-20°C	8	+ 2,7 ± 3,1	+ 4,5 ± 6,3	+ 4,1 ± 2,0

*Changes in plasma ammonia concentrations expressed as measured concentration minus baseline concentration

deamination of proteins like glutamine or due to breakdown of adenyl pyrophosphate and/or adenylic acid[10]. However, this study has shown that equine plasma can be stored at -20°C for up to 24 h before the ammonia determination is carried out.

Although values from the present study can be used as a reference, it is advisable that each laboratory determine its own base-line values, due to inter-laboratory variability. The clinical usefulness of the ammonia tolerance test to diagnose congenital or acquired portocaval shunts in horses, needs to be determined.

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